

Shin WATANABE*: The genus *Chlorella* (Chlorococcales)
from Japanese soils (3)**

渡辺 信*: 日本産土壌クロレラ属
(クロロコックム目) (3)**

Chlorella zofingiensis Dönn 1934, p. 127; Fott et Nováková 1969, p. 42,
pl. 13. (Fig. VI)

Adult cells spherical; young cells spherical, subspherical or ellipsoidal. Adult cells 7.0–12.0 μm in diam., rarely up to 17.0 μm in diam.; young cells 3.0–3.5 $\times$ 4.0–5.0 μm in size; autosporangium up to 23 μm in diam. Cell wall thin throughout culture. Chloroplasts in young cells saucer-shaped, broadly band-shaped or girdle-shaped, being incised with growth; those in adult cells parietal with deeply, irregularly cleft margin, occupying the most part of cell's periphery, sometimes segmented in old culture. Pyrenoid lacking. Starch accumulation sometimes present. Nucleus and nucleolus rarely visible without stain. Vacuoles absent. Oil droplets present.

Reproduction by 2–64 autospores. Autospores equal in size in a sporangium. Empty mother-cell wall pouch-like with a sharply defined opening.

Plant mass smooth, green, turning to brownish orange in old cultures.

Culture No. SAI-22, SAI-27, SAK-17, KUC 17-4, HKN-29.

Within the species there were recognized two groups which were, however, not separated as varieties. One group consisted of isolates, SAI-27, KUC 17-4 and HKN-29 (Fig. VI, 1–8) coincides with the descriptions of Dönn (1934) and Fott and Nováková (1969). Another one of SAI-22 and SAK-17 (Fig. VI, 9–18) is somewhat different from the former in the morphology. Although *C. zofingiensis* has been said not to accumulate starch, two isolates of the latter group exhibited some dark blue starch granules in the chloroplast when they were stained with I₂KI. According to the observation of minute granules in the former group without stain, starch-like compounds might be formed also in these isolates. The chloroplasts of young cells in the

* Laboratory of Applied Botany, Department of Agriculture, Kyoto University, Kyoto, 606.
京都大学農学部応用植物学研究室.

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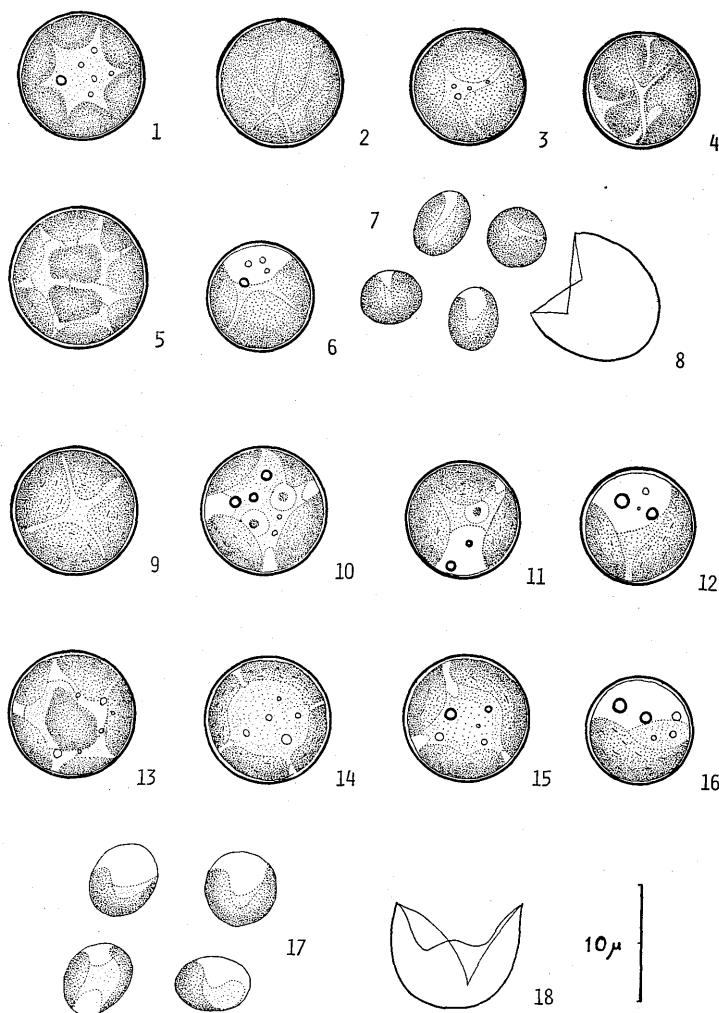


Fig. VI. 1-18 : *Chlorella zofingiensis* Dönn. 1-8, isolates lacking the starch accumulation. 1 and 3, spherical cells with a parietal chloroplast. 2 and 4, surface view of cell; with deep and irregularly branched lobes. 5, old segmented chloroplasts. 6, young vegetative cell with a chloroplast which occupies not so large portion. 7, just liberated autospores. 8, empty mother-cell wall. 9-18, isolates accumulating stroma starches. 9, spherical cell with a parietal and typical zofingiensis-type chloroplast. 10, 11, 14 and 15, spherical cells with a parietal chloroplast which is incised at several points. 12, young spherical cell with a band-shaped chloroplast. 13, old cell with segmented chloroplasts. 16, young cell with a saucer-shaped chloroplast which occupies about a half of cell's periphery. 17, just liberated autospores. 18, empty mother-cell wall.

former group are girdle-shaped, and those of adult cells are parietal to occupy the almost entire portion of the cell's periphery. On the other hand, the chloroplasts of young cells in the latter are saucer-shaped or broadly band-shaped to occupy 2/3-3/4 of the cell's periphery. In the latter, the typical shapes of the plastid of *C. zofingiensis* were observed in some adult cells only. The nucleus and nucleolus were sometimes observed without stain in the cells of SAK-17.

It is questioned if the latter group is identical with *Lobosphaera tirolensis* Reisinger (1964). In the morphology of the chloroplast, however, *L. tirolensis*, does not perfectly agree with my isolates, so the identification could not be confirmed.

Chlorella protothecoides Krüger; Chodat 1913, p. 114, fig. 109; Shihira et Krauss 1963, p. 39, fig. 51, 52; Fott et Nováková 1969, p. 44, pl. 14.

(Fig. VII, 1-12)

Adult cells spherical; young cells and autospores ellipsoidal to spherical. Adult cells 4.0-6.0 μm in diam.; young cells and autospores 3.0 μm in diam. or 2.0 $\times$ 2.5 μm in size. Cell wall thin throughout culture. Chloroplast broadly band-shaped or cup-shaped, occupying up to half of cell's periphery. Pyrenoid and starch accumulation lacking. Nucleus and nucleolus invisible without stain. Vacuoles absent. Oil droplets present, increasing with age.

Reproduction by 2-4 autospores. Autospores equal in size in a sporangium. Empty mother-cell wall pouch-like and readily modified.

Plant mass smooth, green, turning to yellow, yellow-green in old cultures. Culture No. OOD-2.

The dimension of cells of OOD-2 is much smaller than that of *C. protothecoides* which was measured by Chodat (1913), Shihira and Krauss (1963), and Fott and Nováková (1969), but this isolate is identified to *C. protothecoides* in that the chloroplast is broadly band-shaped or cup-shaped, occupying up to one-half of cell's periphery.

Chlorella minutissima Fott et Nováková 1969, p. 50, pl. 15, o-y.

(Fig. VII, 13-21)

Cells spherical; young cells and autospores somewhat ellipsoidal. Cells 2.0-4.0 (-7.0) μm in diam. Cell wall thin throughout culture. Chloroplast cup-shaped, girdle-shaped, or mantle-shaped, occupying 2/3-4/5 of cell's periphery. Pyrenoid and starch accumulation lacking. Nucleus and nucleolus

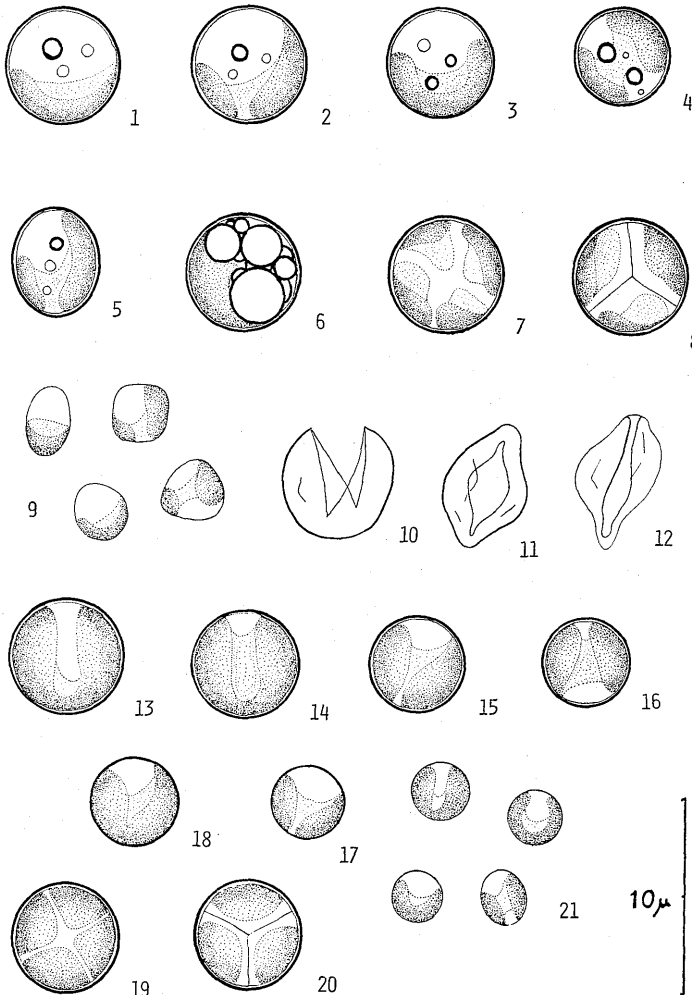


Fig. VII. 1-12: *Chlorella protothecoides* Krüger. 1, spherical cell with a saucer-shaped chloroplast. 2 and 4, spherical cell with a band-shaped chloroplast. 3, spherical cell with a cup-shaped chloroplast. 5, ellipsoidal cell with a broadly band-shaped chloroplast. 6, cell in old culture with abundant oil droplets and a retired chloroplast. 7, cell with divided chloroplasts. 8, cell with just formed dissepiments. 9, sub-spherical, just liberated autospores. 10-12, empty mother-cell walls.

13-21: *Chlorella minutissima* Fott et Nováková. 13 and 14, spherical cell with a mantle-shaped chloroplast. 15-17, spherical cell with a broadly band-shaped chloroplast. 18, spherical cell with a girdle-shaped chloroplast. 19, spherical cell with divided chloroplasts. 20, cell with just formed dissepiments. 21, just liberated autospores.

invisible without stain. Vacuoles lacking. Oil droplets present.

Reproduction by 4 autospores. Autospores formed by progressive cleavage of protoplasm, equal in size in a sporangium. Empty mother-cell wall not observed.

Plant mass smooth, green, not changing in color in old cultures.

Culture No. YAW-119.

It is originally described that the distinct characteristic of the species is the small dimension of cells, that is, up to $3.5\ \mu\text{m}$ in diam. under favourable conditions. When YAW-119 was isolated from soil, its larger cells were $4.0\ \mu\text{m}$ in diam. and rarely reached $6.0\ \mu\text{m}$ in diam., but after many times of transfers of the alga for about 4 years, it was observed that cells were ordinarily $6.0\ \mu\text{m}$ in diam. at the largest size and rarely reached $7.0\ \mu\text{m}$ in diam. These larger dimensions of cells resemble those of OOD-2, which is identified to *C. protothecoides*. However, YAW-119 was identified as *C. minutissima* by the morphology of the chloroplast, that is, cup-shaped, girdle-shaped or mantle-shaped, occupying $2/3$ - $4/5$ of cell's periphery.

Chlorella reniformis Watanabe sp. nov. (Fig. VIII)

Cellulae dispositae in duobus typis; cellulae E-typi elongate cylindricae, ellipsoideae vel reniformes; et cellulae S-typi sphaericae vel subsphaericae. Cellulae E-typi 2.0 - 10.0×4.0 - $14.0\ \mu\text{m}$; et cellulae S-typi 5.0 - $17.0\ \mu\text{m}$ diametro. Membrana tenuis. Chloroplastus cellularum iuvenium E-typorum vittiformis vel acetabuliformis, circumiens circa $1/3$ peripherae cellulae; S-typorum et adultarum E-typorum olliformis cum subtilis apertura vel profunde lobatus, circumiens fere integeram partem peripherae cellulae. Pyrenoides et cumulus amyli absentes. Nucleus et nucleolus interdum visibiles. Vacuolae interdum praesentes. Guttulae olei suppetentes.

Reproductio 2-16 autosporis. Autosporae E-typorum ubi (8-)16 sporae in sporangio factae; et S-typorum ubi 2 vel 4 sporae in sporangio factae. Membrana matris profunde rumpit, facile mutata.

Massa plantae laevis, viridis, non mutans colore in cultura vetere.

Numerus culturae: KIY-21, KIY-25, KUC 25-1, OOD-14, IWA-15.

The shape of cells is classified in two types; E-type cells are elongate-cylindrical, elongate-ellipsoidal or elongate-reniform; and S-type ones are spherical or subspherical. These two types of cells are determined according to the number of autospores formed in a sporangium. The occurrence of

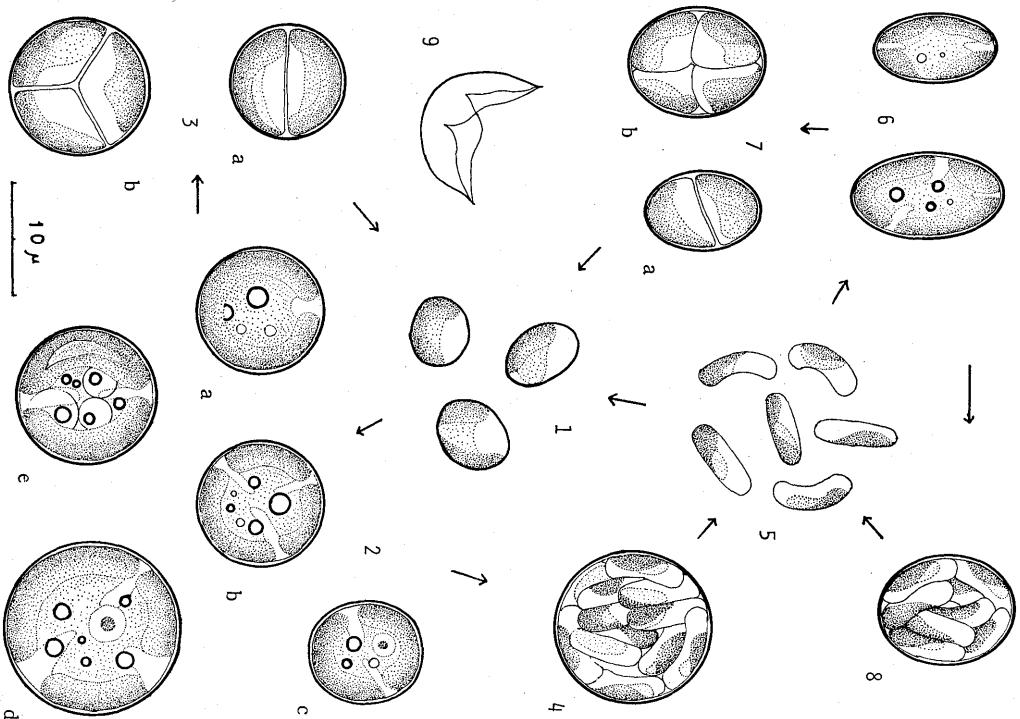


Fig. VIII. 1-9: *Chlorella reniformis* Watanabe sp. nov. 1, young S-type cells. 2, adult S-type cells: a, with a mantle-shaped chloroplast; b-d, with a deeply cleft chloroplast; and e, with a deeply cleft chloroplast which is cracked to form crescent-shape, and with vacuoles. 3, S-type sporangium containing S-type autospores; a, 2 autospores; and b, 4 autospores. 4, S-type sporangium containing E-type autospores. 5, young E-type cells. 6, adult E-type cells. 7, E-type sporangium containing S-type autospores; a, 2 autospores; and b, 4 autospores. 8, E-type sporangium containing E-type autospores. 9, empty mother-cell wall. Arrows indicate the order of the cell growth.

the two types in the life cycle coincides with that of *C. reisiiglii*. E-type cells $2.0-10.0 \times 4.0-14.0 \mu\text{m}$ in size; and S-type cells $5.0-17.0 \mu\text{m}$ in diam. The cell wall is thin throughout culture. The chloroplasts in young E-type cells are band-shaped or saucer-shaped, occupying about $1/3$ of cell's periphery. They enlarge and become mantle-shaped in S-type and adult E-type cells to occupy almost entire portion of cell's periphery. The margin of the chloroplasts in young E-type cells are smooth and relatively simple, whereas those of adult cells are deeply cleft at two or three points, sometimes to make a bridge. It was often observed that a part of the chloroplast of OOD-14 was cracked to form crescent-shape. The cracked portion was colored in pale green and became larger with age. At the present observation with light microscope, it is not confirmed to be a pyrenoid. Starch accumulation was not detected by the stain with I_2KI . In IWA-15 and OOD-14 the quantity of vacuoles and oil droplets was sometimes abundant and dimensions of cells were larger than those of other isolates. However, the fact that the vacuoles did not occur abundantly in every cultural occasions and their size was not far beyond those of others, leads to inclusion of these isolates in the species.

Reproduction is made by the formation of two types of autospores. E-type autospores are formed when spores are (8-) 16 in number in a sporangium; and S-type ones are formed when 2 or 4 in number. The mother-cell wall deeply breaks down and is modified easily.

The plant mass is smooth and green, not changing in color in old cultures.

Discussion

The habitat of *Chlorella* is summarized by Fott and Nováková (1969) as follows: freshwater or marine, some species aerial, growing in soil, on humid rocks, bark, wood, etc., sometimes endozoic. In the present study, many species of *Chlorella* have been isolated from soils very frequently. It is evident that *Chlorella* is ecologically an important member of soil algae.

In this paper, 12 taxa of *Chlorella* are recorded according to the generic definition by Beijerinck and Fott and Nováková who include species with and without pyrenoids in a genus. These authors regarded the existence of pyrenoid as a specific criterion in the genus *Chlorella*, and Fott (1971) consistently considered so in the other taxonomic groups. On the other

hand, pyrenoids are considered to be a generic criterion as seen in some orders by many taxonomists, including Gerloff (1962), Starr (1955), Deason (1967), Herndon (1958), Vischer (1933) and Bold (1970). These two different evaluation of pyrenoid in the taxonomy are inevitably due to the fact that the function of pyrenoids is not fully known. Of the criteria used in the taxonomy at the rank of species in this paper, the presence or absence of pyrenoids is qualitatively different from other characters. Although a direct evidence is not given, it could be presumed that the pyrenoids reflect a different stage in the evolution of chloroplast (Klein and Cronquist 1967). Then the presence or absence of pyrenoids is not quantitatively comparable. Other features such as cell sizes and shapes of chloroplast are, however, quantitatively comparable. Therefore, the pyrenoid seems to provide a basis for a separation of the genus at least.

The genus *Chlorella* has hitherto been studied mostly by light microscope. In the case that pyrenoids are clearly surrounded by starch grains, their presence is readily observed. However, as shown in *C. saccharophila* var. *saccharophila* whose pyrenoid is not surrounded by starch. It is very difficult to discriminate it. Two algae which are not reported in this paper were isolated from Kyoto: although the isolates closely resemble *C. saccharophila* var. *saccharophila* in the cell shape and size, they could not be identified to it because the presence of pyrenoid was not recognized by light microscopy. In this connection, it should be pointed out that *Stichococcus*, a unicellular Ulotrichalean genus, though hitherto considered to lack pyrenoid, has been revealed to possess pyrenoid by electron microscopy by Pickett-Heaps (1974) and Silverberg (1975). In the genus *Chlorella*, it may be possible that the pyrenoid which cannot be observed with light microscope is discovered with electron microscope. Chodat (1894) established the genus *Palmellococcus* which is distinguished from the genus *Chlorella* in the absence of pyrenoid. Five *Chlorella* species which are considered to lack pyrenoid have been described to date. When the absence of pyrenoid in these species is confirmed by the observation with electron microscope, they will be transferred to the genus *Palmellococcus*.

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日本各地の土壌から分離したクロレラ属の藻株 34 を主として Fott & Nováková (1969) の分類系に基づいて研究した結果、9 種 2 変種 (うち 1 種は新種) を同定した。そのうちの 5 種 2 変種は既に第 1 報と第 2 報に記述した。本報告では *Chlorella zofingensis*, *C. protothecoides*, *C. minutissima* 及び *C. reniformis* の 4 種を記載した。最後に挙げた種類は今回新種として記載したものである。

本論文ではピレノイドの有無にかかわらずすべての種をクロレラ属に所属させたが、ピレノイドの有無を生物学的に検討することにより、ピレノイドを欠く数種のバルメロコックス属への移行の可能性についても論じた。

□寺崎留吉・奥山春季編：寺崎日本植物図譜 四六倍版 1083+41+38 pp. 平凡社、東京 (1977, V.) 8,000円。Terasaki's Illustrated flora of Japan, Heibonsha, Tokyo. 日本植物を主とし、これに帰化植物、栽培植物を加え、さらに南方で普通の重要植物を加えて網羅した図譜がいよいよ出版された。さきに 1933 年及び 1938 年の 2 回にわたって出版された 4,000 図に、未発表の 1,000 図を加えた中から選び直して、これに 300 種を加えて 4,317 種を一挙に掲げた図譜である。原図は寺崎さん一人が 50 年に亘って、現地を主にして実物を前にして毛筆で描き上げたものであるから、まことに生彩を放っている。対面の 2 ページに主に 7 または 8 図づつを配して、その余白にその解説を新たに作り、必ずそのページで納まるように工風がしてあるのもよい。順序は主にエングラのシステムに従っている。非常に筆の早やかった寺崎氏の筆のなるだけに、どのページを開いても潑刺としたその植物の姿態が目にとび込んで来て、気持がよいのも嬉しい。スミレ (53), サクラ (37), タケ (35), ヤシ科 (29), カンアオイ (13) という風に数多くの植物があげられ、一々適切な図と解説がなされている。花と果実とでひどく違うものは花、実 2 つの図を挙げ、ソテツやソゴは雌雄で 2 図にもなっている。

恐らく今後このような一人の人の腕になる図をもとにした図鑑は、もう出版されないと考えると、この改版増補はたしかに意義があったと思われる。それにトウテイランやセンブリなどには他の図書と異なる新しい学名が使われているのも注意を要する。

(前川文夫)